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From: Lawler, Michael (DPH)
Sent: Thursday, November 18, 2010 6:47 PM
To: Nassif, Julianne (DPH); Salemi, Charles (DPH); Tan, Zhi (DPH); Piro, Peter (DPH)
Subject: clarification of the rationale for concetrating GHB samples in the most recent prep

Please note from the protocol that the analysis of GHB in unknown liquids is accomplished with as many as three HPLC runs. In the initial run we determine whether the sample is ideal, dilute or concentrated, based on the appearance of a signal at the Rt of GHB.

In most cases the first run reveals that 50 to 75% of the samples have to be adjusted to drive the suspect GHB response above the level of the noise. I believe in most cases this noise would be sucrose or corn syrup, the most preponderant additives to soft drinks used as mixers.

As the concentration is adjusted for each HPLC run, suspect GHB appears as an early eluting shoulder on the "sweetener" signal. The method we use tweaks this shoulder out of the noise. But it takes three runs to resolve this for a typical cluster of samples. As a rule of thumb, most samples have to be *concentrated* rather than diluted to muscle out the suspect GHB shoulder.

I was delighted to have the opportunity to use the HPLC in the Environmental Lab to assay the materials submitted by Braintree. However, I was anticipating using the device for several days, for the usual three runs titrating my samples for the strongest signal. Anticipating running the first set Thursday the 18th, I inquired when I might do the second run. I had hoped to do the remaining runs in a timely manner before the material decayed; that is, within an interval of two or three additional days. This would have presented the most likely candidate samples for Mr. Piro's analysis, which would have retired the case. To my dismay, I learned that any second run could only be done in January, after December's CDC validation studies.

This of course would render all my preps and attendance to protocol moot for the fifth time this year, in light of the four catastrophic failures of the old machine the Drug Lab had in place. One run would have been meaningless. With the knowledge that the second HPLC run is the most informative and that only after most of the samples have been concentrated, I decided to prepare the samples in a manner which would most likely mimic the second run; that is, I concentrated them. I would have you all note this was not a "lab mistake." Rather, I conscientiously prepped the samples in a manner which would give the most information for the one run I was afforded.

Ironically, after that run was completed I was offered an additional run. So be it. I used the first run data to best titrate the samples for the next run, diluting some while concentrating others in the usual manner. Despite the concerns for the level of background noise in these samples, I believe these runs have revealed the most likely candidates for GHB from this sample pool. The next step is to present them derivatized to Mr. Piro for him to discern whether any *suspect* peaks are indeed GHB.

It is wonderful that we now have an entire team working on the resolution of the HPLC response and finding routes for cleaning up the samples. From my own experience and literature searches over the past two years, I would posit that the most fruitful cleanups to consider would be those which may eliminate sucrose or corn syrup, without the use of an alkali system and most certainly without raising the ambient temperature of the samples above 70 degrees C.